Section 1 - Publishable summary

EPITARGET

Logo: [EPITARGET logo]

Project title: Targets and biomarkers for antiepileptogenesis
Website: www.epitarget.eu

Contractors involved (EPITARGET consortium):
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1.1 Summary description of project context and objectives

EPITARGET is a large European consortium of universities, research institutions and SMEs dedicated to understand complex mechanisms and identify biomarkers of epileptogenesis to improve treatment and diagnosis of epilepsy. The overall scientific objectives of EPITARGET are:

Using multidisciplinary strategies of basic, preclinical and clinical research to

1. identify novel biomarkers and their combinations that will define the different stages of epileptogenesis and predict/diagnose early and late stages of the evolution of the disease. This will pave the way to improved diagnostics and better patient stratification, as well as development of patient-specific preventive strategies. In this objective EPITARGET will identify at least 2 biomarkers that in combination will predict whether epileptogenesis is triggered in post-insult period and will stratify those individual subjects that are at risk of developing epilepsy.
2. unravel the complex patho-physiology of epileptogenesis and to design new, disease-modifying combinatorial treatment strategies specifically targeted to the different stages of epileptogenesis. These novel treatment strategies are expected to be capable of preventing the development of epilepsy in at-risk patients and stop its progression after the onset of the disease. Different epileptogenesis-associated processes, such as brain damage and structural/functional reorganization, neurogenesis, BBB dysfunction, inflammation, free radical formation, genetic and epigenetic alterations will be individually addressed and specific treatments directly or indirectly targeting these mechanisms will be combined. The choice of the best combinatorial therapeutic strategy will be assisted by systems biology approaches. This strategy will help to identify key aetiological factors thus limiting redundancy in the choice of multiple drug targets and facilitating rational drug discovery process. In this objective EPITARGET will identify at least 2 patho-physiological mechanisms of epileptogenesis that will be targeted in combination in animal subjects at risk of developing epilepsy (post-insult period).

3. translate the knowledge obtained in experimental models to patients in order to improve diagnosis, achieve better patient stratification, and develop new antiepilepticogenic treatments and means to predict their efficacy. The objective is to verify and validate biomarkers in blood and brain tissue samples obtained from patients after potentially epileptogenic brain insults. These tissues include TBI and post-mortem brain tissue from patients that had SE or TBI (early-stage epileptogenesis), as well as from those with chronic epilepsy. These specimens are the best closest match to the experimental brain tissue of post-insult epileptogenesis in animal models. In this objective EPITARGET will perform clinical validation of the combinatorial biomarker approach (objective 1), and thereby obtain data from patients to prove the concept for future clinical applications.

To achieve these objectives and maximise outcome, EPITARGET tackles the complexity of epileptogenesis by adopting concerted and complementary actions of participating partners, attacking various aspects of epileptogenesis at different levels. EPITARGET combines a powerful arsenal of both established and innovative multidisciplinary research strategies, tools and platforms. Analysis of data are assisted by creating an animal and human database, and a bioinformatics approach within the consortium.

1.2 Work performed since the beginning of the project and the main results achieved so far

WP1
EPITARGET consortium developed Epilepsy Preclinical Biomarker Bank Case Report Forms containing the Common Data Elements and Guidelines for procedures of experimental work. The CDEs are now published on the EPITARGET webpage, available for scientific community.

Subset of miRNAs in the brain tissue is altered at certain time-points during the epileptogenesis in different animal models, suggesting that they may be disease-specific rather than model-specific. Some miRNAs are altered in plasma and may be proposed as putative biomarkers of epileptogenesis.

Some inflammatory biomarkers were monitored using positron emission tomography, enabling calculation of neuroinflammation time course, peaking at 1-2 w after status epilepticus (SE). Dual-labelled serum albumin tracer has been also developed to monitor blood-brain barrier leakage during epileptogenesis.

MRI protocol containing T2-T2* mapping, phase-imaging, and high angular and spatial resolution DTI has been tested. Oscillating field diffusion MRI was shown to detect changes in cell density in hippocampus after SE with high specificity and sensitivity.

WP2
Pro-resolving inflammatory signalling is activated in human pharmacoresistant epilepsy. In animals, preventing oxidative stress reduces cell loss during epileptogenesis and delays epilepsy onset. Thus, drugs mimicking pro-resolving molecules or reducing oxidative stress may act as potential anti-epileptogenic treatments.

Certain miRNA-mimic treatment post-SE reduces cell loss and improves memory deficits, suggesting some inflammatory signalling as a putative target for neuroprotection, and resolving co-morbidities during epileptogenesis.

Immunoproteasome (IP) subunits are overexpressed during epileptogenesis in rodent models and in resected brain tissue of TLE patients. Some compounds attenuated gene expression of the IP subunits and reduced seizure activity. \textit{in vitro}.\i
Microglial cells increase their interaction with newborn hippocampal neurons during a particular developmental window and at certain subcellular regions following SE. Microglia activation and astrocystosis arise in the retina following SE, thus highlighting a potential biomarker of neuroinflammation.

Tolerable combinations of clinically available drugs were designed and are studied for anti-epileptogenesis. The mouse and rat models for the two-step approach of drug testing have been set up. Two rat SE models have been refined for improved biomarker search.

A combination of phenotypic biomarkers, including seizure threshold and behavioral hyper-excitability, are predictive of epilepsy development in the lithium/pilocarpine model in rats.

WP3

To target therapeutic molecules into the brain, more stable amplicon vectors were developed, which were tested in mouse hippocampus for long-term expression of the transgene. The SME Bioviron is working to establish the best plan towards translational therapeutic applications.

WP4

EPITARGET established the ‘virtual human brain tissue database’, containing very dense and high quality clinical and neuropathological data.

Systems genetics approaches characterized the genetic regulation of pathophysiological pathways in human TLE. Using resected hippocampi from pharmacoresistant TLE patients, we identified a gene-regulatory network that contains a specialized, highly expressed transcriptional module encoding pro-convulsive cytokines and Toll-like receptor signalling genes.

Complementary RNA sequencing analysis in a mouse model of TLE demonstrated that the pro-convulsive module is specific to the epileptic hippocampus in animals, suggesting preservation across the species.

Morpholino-mediated knockdown in a zebrafish epilepsy model confirmed the regulation of the transcriptional module, and attenuated chemically induced behavioral seizures in vivo.

WP6

Major results for dissemination were the generation of a definitive review paper, the organization of the 1st EPITARGET Young Researchers’ Symposium and the launch of the EPITARGET Facebook.

1.3 The expected final results and their potential impact and use (including the socio-economic impact and the wider societal implications of the project so far)

The overall expected final results of EPITARGET are to (i) identify combinatorial biomarkers for epileptogenesis, diagnostics, prediction of disease progression and pharmacoresistance; (i) identify combinatorial preventive and disease-modifying treatments targeting complex pathophysiological mechanisms of post-insult epileptogenesis; and (iii) validate combinatorial biomarkers from animal studies in human subjects and tissue. These results are envisaged to have a dramatic impact on the socio-economic burden of epilepsy by improving quality of life for millions of people and their families, reducing the costs related to epilepsy, currently estimated as 20 billion € for Europe only. Societal implications of the final results of EPITARGET are expected to have significant influence on a wide range of areas including patient and professional organizations, professionals in clinical practice, regulatory authorities, pharmacological industries and SMEs, funding organizations, other brain diseases, as well as on health status of the general population.

Objective 1:

Establishing Epilepsy Preclinical Biomarker Bank (EPBB) and database will have an impact not only on the outcomes of EPITARGET, but will have wider implications for the epilepsy research community, as well as for other areas of neurological diseases. This will be one of the first animal biobanks and databases that will lay a foundation for other disease model biobanks, facilitating the preclinical research. CDEs and CRFs already developed by EPITARGET will be valuable for ILAE task force dedicated for this mission, as well as for other consortia in epilepsy established in USA by NINDS initiative for Centers Without Walls (C WOW). EPITARGET CDEs are now available to the general public and research communities on the EPITARGET webpage.

Objective 2:
EPITARGET has already generated data on temporal and spatial patterns of gene and protein expression for a wide range of factors and molecules potentially involved in epileptogenesis, which is broadening our understanding of epileptogenesis, and eventually will result in developing combinatorial antiepileptogenic treatment strategies.

Results obtained during first years of EPITARGET in evaluating combinatorial treatment approaches based on existing drugs will have an impact on shifting the paradigm of thinking about possibilities for preventive and disease modifying treatment strategies after brain insults. Novel drug combinations are also generating results and will increase our knowledge and understanding of antiepileptogenic treatment possibilities. Moreover, results on optimization and standardization of post-SE animal models are very useful not only for EPITARGET but the whole epilepsy research community, and will contribute for more understanding of those factors, such as animal strain and batch variability, SE duration and termination procedures, site of insults of the brain, etc., in contributing variable outcomes of the preclinical studies.

Large capacity Amplicon viral vectors and lipid encapsulation approaches that are validated by EPITARGET will open novel avenues for combinatorial treatment approaches in preventing epileptogenesis and disease progression, and contribute to innovative thinking and exploitation of the results for commercialization strategies of SMEs.

Objective 3:
Ongoing prospective collection of samples from brain tissue, imaging, EEG and fluids from TBI and epilepsy patients will have a crucial role in securing validation of animal data in human material, paving foundation for translational research and clinical applications. Inclusion of patient data, and establishment of sample collection logistical details secures timely progress of validation process envisaged by EPITARGET. This material can also be used for data mining and systems biology approaches, and may also lead to human biobank development in the future, which will have significant impact on the research possibilities even outside EPITARGET.